Bull. Org. mond. Santé }
Bull. Wld Hlth Org. } 1969, **41**, 915-928

Chemosterilization of *Culex pipiens fatigans* Wiedemann by Exposure of Aquatic Stages

1. Sterilization Potential of Certain Aziridinyl Compounds

M. K. K. PILLAI [1] & K. K. GROVER [2]

The sterile-male technique has been considered to be a promising tool for the control of mosquito populations. Many chemosterilants are known to sterilize insects effectively in the same way as ionizing radiations. The sterilizing activity of 8 alkylating aziridinyl compounds has been assessed on the tropical house-mosquito, Culex pipiens fatigans *Wiedemann. The chemosterilants employed for larval and pupal treatments were apholate, metepa, tepa and 5 diaziridinyl alkoxyphosphine oxides. In general, metepa was least toxic to the various life stages of* C. p. fatigans *during the treatment. Apholate was toxic only in pupal treatments while tepa was less toxic in pupal treatments and more toxic in larval treatments. Diaziridinyl compounds were moderately toxic in both larval and pupal treatments. The tepa and apholate treatments caused certain structural and moulting abnormalities in pupae and adults and also induced abnormalities in eggs and larvae.*

Among the polyaziridinyl compounds, non-toxic doses of apholate could produce about 100 % sterility with larval treatment. Tepa was inferior to apholate, and metepa was least effective in larval treatment. Among the diaziridinyl compounds, the methyl ester was most promising. For pupal treatment, tepa was more effective than apholate and metepa at non-toxic doses, inducing almost complete sterility. The propyl and isopropyl esters of the diaziridinyl compounds also induced very high sterility in pupal treatments.

The spectacular success of eradicating the filariasis vector, *Culex pipiens fatigans* Wiedemann in Okpo, Burma, by release of incompatible males into the natural population has once again reinforced our faith in the potentialities of the sterile-male-release technique for the control of this vector as well as other mosquitos, although earlier attempts in this direction were not encouraging (Laven, 1967; Morlan, McCray & Kilpatrick, 1962; Weidhaas, Schmidt & Seabrook, 1962; Krishnamurthy, Ray & Joshi, 1962). Sterility in insects can be induced by irradiation or by chemosterilization and in theory the latter method has been shown to supersede both insecticide treatment and irradiation methods (Knipling, 1959; Bořkovec, 1966; Smith, 1967). So far chemosterilants containing aziridinyl functional groups have been the most effective compounds and among them the aziridinyl phosphine oxides are particularly outstanding (Bořkovec, 1964; Chang & Bořkovec, 1966). Laboratory screening studies on the effect of these alkylating chemosterilants have shown encouraging results against *C. p. fatigans* (Mulla, 1964; Das, 1967; Grover, Pillai & Dass, 1967). This encouraged us to commence further studies of 1 hexaaziridinyl, 2 triaziridinyl, and 5 diaziridinyl compounds to assess their effectiveness in inducing sterility in *C. p. fatigans*.

MATERIALS AND METHODS

The strain of *C. p. fatigans* employed for the present investigation originated from a field strain collected near Delhi in 1965 and maintained since then in the laboratory at 26.7°C and 80% (±5%) relative humidity. The 8 chemosterilants [3] used in

[1] Reader in Zoology, Department of Zoology, University of Delhi, Delhi-7, India.

[2] Research Scholar, Department of Zoology, University of Delhi, Delhi-7, India.

[3] Samples of the chemosterilants were kindly provided by Dr A. B. Bořkovec, US Department of Agriculture, Md., USA.

the present studies may be grouped into 3 categories as follows, based on the number of aziridinyl rings in each compound:

(1) Hexaaziridinyl compound: apholate—2,2,4,4,6,6-hexahydro-2,2,4,4,6,6-hexakis (1-aziridinyl)-1,3,5,2,4,6 triazatriphosphorine.

(2) Triaziridinyl compounds: tepa—tris (1-aziridinyl) phosphine oxide; and metepa—tris (2-methyl-1-aziridinyl) phosphine oxide.

(3) Diaziridinyl compounds: in this series, 1 aziridine ring has been replaced with a series of alkoxy groups in the parent compound tepa to produce the following compounds:

(*a*) Phosphonic acid, bis(1-aziridinyl)-methyl ester,

(*b*) Phosphonic acid, bis(1-aziridinyl)-ethyl ester,

(*c*) Phosphonic acid, bis(1-aziridinyl)-propyl ester,

(*d*) Phosphonic acid, bis(1-aziridinyl)-butyl ester, and

(*e*) Phosphonic acid, bis(1-aziridinyl)-isopropyl ester.

Toxicity of chemosterilants

The toxic effects of apholate, tepa and metepa on 2nd-, 3rd- and 4th-instar larvae and pupae were separately assessed by treating 100 larvae in 500 ml, and pupae in 20 ml, of an aqueous solution of the chemosterilants in graded concentrations. The larvae were exposed continuously until pupation, while the pupal treatment lasted for 28 h. The experiments were replicated twice. During the treatment the larvae were fed on a mixture of brewers' yeast and blood albumen (5 : 1). Controls were maintained under identical conditions. Mortality counts were made at intervals of 24 h until the adults had emerged. Percentage mortalities were calculated from the data separately for larval, pupal and adult stages. The effects of chemosterilants on moulting and other structural abnormalities were also assessed. In the case of the diaziridinyl compounds the toxic effect was determined with only those concentrations that were used in the larval and pupal sterility treatments.

Sterility by larval treatment

Early 2nd-instar larvae were exposed to various concentrations of the chemosterilants in distilled water. In each experiment 200 larvae were used and the treatment continued until pupation. During treatment the larvae were fed on brewers' yeast and albumen. The newly emerged pupae were washed in distilled water and transferred for emergence to small vials containing water. Newly emerged adults were immediately sexed and used for mating experiments. The following crosses were made (*a*) treated males × treated females, (*b*) treated males × normal females, (*c*) normal males × treated females and (*d*) normal males × normal females. In each cross, 20 males and 20 females were placed in 18 in × 16 in × 16-in (45 cm × 40 cm × 40 cm) screened mosquito cages. Adults were provided with 1% glucose solution in soaked cotton pads and females were given a pigeon-blood meal on alternate days. Egg-rafts were laid in enamel bowls provided with water. To calculate the oviposition rate and egg hatchability the egg-rafts collected in the first 15 days after the start of the experiment were used.

Throughout this paper the term " sterility " is used to mean the infertility or non-hatchability of eggs; thus high sterility means low egg hatchability. " Fecundity " refers to the ability to lay eggs.

Sterility by pupal treatment

In another series of experiments, 200 newly emerged pupae of each sex were exposed to various concentrations of the chemosterilants in distilled water for a period of 28 h. Then they were washed in clean water and later transferred to small vials for emergence. Newly emerged adults were employed in crosses as in the case of the larval treatment. Parallel control experiments were also performed using the same number of pupae. Fecundity and sterility were assessed as in the earlier experiments.

In both series of experiments the control of reproduction was calculated using the formula of Chamberlain (1962). In experiments with diaziridinyl compounds, crosses were made only between treated males and treated females.

RESULTS

Toxicity of the chemosterilants

The toxicity of apholate, tepa and metepa at the different life stages of *C. p. fatigans*, exposed as 2nd-, 3rd- and 4th-instar larvae and pupae to various concentrations are given in Tables 1, 2 and 3 respectively. In all the larval treatments 10 ppm of apholate was almost non-toxic to *C. p. fatigans* (Table 1). However, 30 ppm and 50 ppm of apholate produced about 100% mortality at different stages. In the

TABLE 1

EFFECT OF APHOLATE ON LIFE STAGES OF *C. P. FATIGANS* EXPOSED AS LARVAE OR PUPAE [a]

Stage treated	Concentration (ppm)	Larval mortality	Pupation	Pupal mortality	Adult emergence	Adult mortality	Corrected total mortality [b]
			Per 100 larvae at the start of the treatment				
2nd instar	5	5	95	4	91	0	1
	10	5	95	5	90	0	2
	15	7	93	18	75	30	51
	20	12	88	37	51	32	79
	30	12	88	68	20	11	90
	50	69	31	31	0	0	100
	100	100	0	0	0	0	100
3rd instar	5	2	98	6	92	0	0
	10	4	96	4	92	0	0
	15	5	95	19	76	26	46
	20	5	95	39	56	32	72
	30	9	91	56	35	19	83
	50	24	76	49	27	27	100
	100	88	12	12	0	0	100
4th instar	5	0	100	4	96	0	0
	10	0	100	1	99	3	0
	15	0	100	2	98	5	3
	20	2	98	5	93	8	11
	30	2	98	9	89	8	16
	50	6	94	36	58	44	85
	100	7	93	88	5	5	100
			Per 100 pupae at the start of the treatment				
Newly emerged pupae	1 000	—	—	4	96	0	0
	5 000	—	—	31	69	3	31
	10 000	—	—	39.5	59.5	1.5	40

[a] Larvae were exposed continuously until pupation, and the newly emerged pupae were exposed for 28 h.
[b] Using Abbot's formula.

larval treatments 10.5 ppm of tepa were more toxic than apholate, causing about 2%–17% mortality at various stages (Table 2). The rate of mortality rose to 58%–100% with tepa at 35 ppm. Metepa was the least toxic among the polyaziridinyl chemosterilants, a dosage of 30 ppm did not produce significant mortality and at 100 ppm metepa produced only about 20%–54% mortality in various larval treatments (Table 3).

It is interesting to note that when they were exposed to these 3 chemosterilants many of the larvae died at the critical moulting period. When treated with 100 ppm apholate at 2nd instar, all the larvae died at 4th instar before pupation. The percentage pupation increased with lower doses of apholate. Tepa and metepa also showed similar effects, suggesting the existence of a relationship between the dose applied and the developmental

TABLE 2

EFFECT OF TEPA ON LIFE STAGES OF *C. P. FATIGANS* EXPOSED AS LARVAE OR PUPAE [a]

Stage treated	Concentration (ppm)	Larval mortality	Pupation	Pupal mortality	Adult emergence	Adult mortality	Corrected total mortality [b]
			Per 100 larvae at the start of the treatment				
2nd instar	3.5	2	98	2	96	0	0
	7.0	2	98	2	96	0	0
	10.5	2	98	12	86	6	17
	14.0	8	92	36	56	6	48
	21.0	18	82	72	10	6	96
	35.0	36	64	64	0	0	100
	70.0	100	0	0	0	0	100
3rd instar	3.5	2	98	2	96	0	0
	7.0	0	100	4	96	0	0
	10.5	0	100	6	94	8	10
	14.0	8	92	10	82	10	25
	21.0	10	90	42	48	24	75
	35.0	36	64	60	4	4	100
	70.0	96	4	4	0	0	100
4th instar	3.5	0	100	0	100	4	0
	7.0	0	100	4	96	0	0
	10.5	0	100	4	96	2	2
	14.0	0	100	12	88	8	17
	21.0	0	100	20	80	16	33
	35.0	10	90	40	50	10	58
	70.0	24	76	66	10	6	96
			Per 100 pupae at the start of the treatment				
Newly emerged pupae	716	—	—	4.0	96	0	0
	3 580	—	—	5.5	94.5	0	1
	7 160	—	—	60	40	2	60

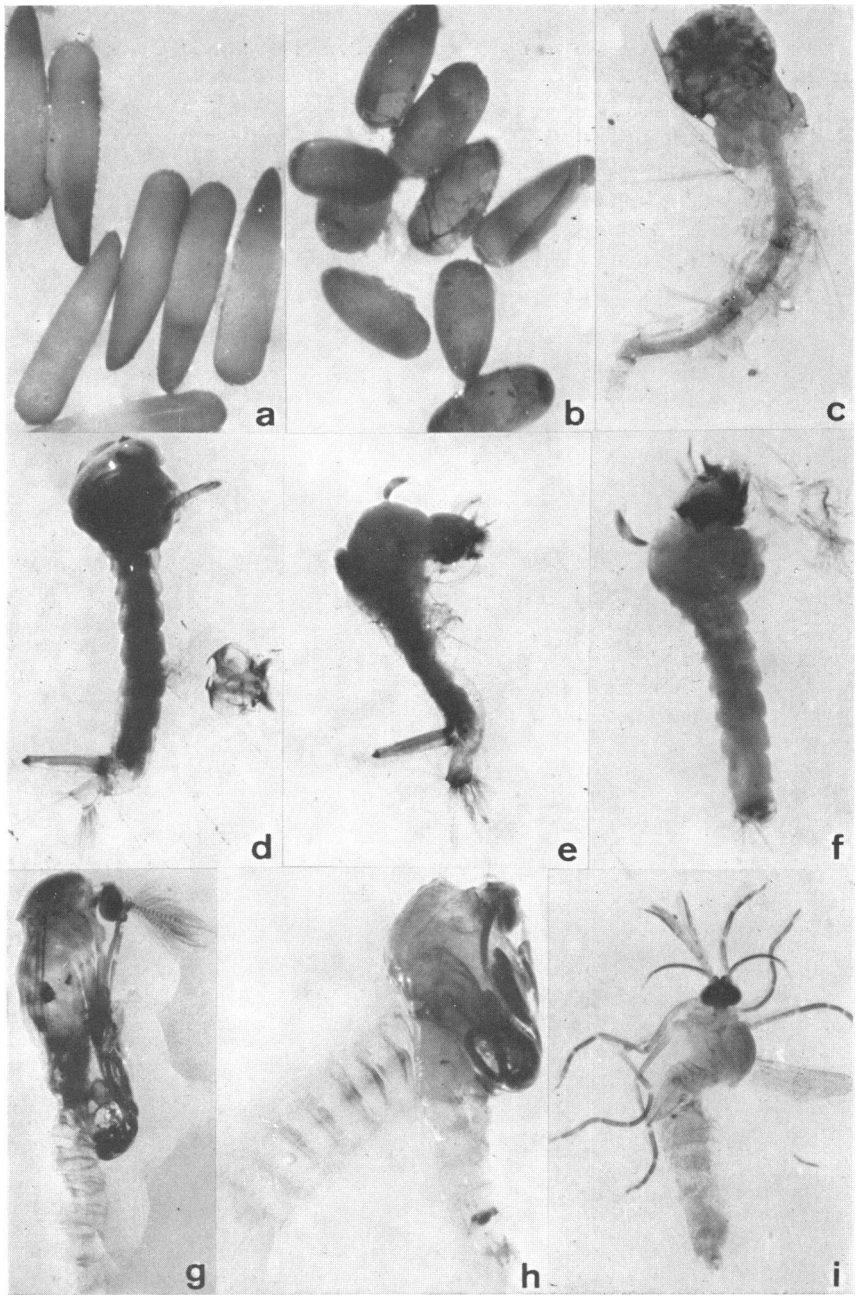
[a] Larvae were exposed continuously until pupation, and the newly emerged pupae were exposed for 28 h.
[b] Using Abbot's formula.

stage at which mortality occurred; the higher the dose, the earlier the developmental stage at which the animal died.

Pupae could tolerate very high doses of all the 3 chemosterilants. Metepa was almost non-toxic at 10 000 ppm and tepa was non-toxic at 3580 ppm. Apholate caused significant mortality at concentrations above 5000 ppm (Tables 1–3).

Among the diaziridinyl compounds, the isopropyl ester was most toxic in the larval treatments with 10 ppm producing about 58% mortality (Table 7). The methyl ester was moderately toxic, 10 ppm causing about 33% mortality, while the others with the same dosage produced only 13%–16% mortality at various stages. When pupae were treated with the diaziridinyl derivatives at 10 000 ppm, the isopropyl and propyl derivatives were the least toxic, giving less than 10% mortality (Table 8 below). At

STRUCTURAL AND MOULTING ABNORMALITIES PRODUCED IN LIFE STAGES OF *C. P. FATIGANS* TREATED WITH TEPA AND APHOLATE (MAGNIFICATION, a–c ×40, d–i ×25)



(a) Normal eggs

(b) Abnormal eggs induced by tepa and apholate treatments

(c) Deformed larva hatched from abnormal egg

(d) Normal moulting of larva into pupa

(e) Moulting abnormality in pupa; moulting started in the middle, pupa with larval head

(f) Moulting abnormality in pupa; moulting started posteriorly and proceeded anteriorly, pupa with larval head

(g) Normal adult emergence from pupa

(h) Abnormality in adult emergence showing the abdomen coming out of pupal moult and the anterior part still inside

(i) Adult male showing abnormalities such as deformed thorax, reduced wings, weak and curved legs, antennae without antennal hairs and deformed abdomen

TABLE 3

EFFECT OF METEPA ON LIFE STAGES OF *C. P. FATIGANS* EXPOSED AS LARVAE OR PUPAE [a]

Stage treated	Concentration (ppm)	Larval mortality	Pupation	Pupal mortality	Adult emergence	Adult mortality	Corrected total mortality [b]
			Per 100 larvae at the start of the treatment				
2nd instar	5	2	98	1	97	2	3
	10	1	99	1	98	3	3
	15	4	96	5	91	3	10
	20	4	96	5	91	10	17
	30	9	91	4	87	7	18
	50	13	87	25	62	11	48
	100	17	83	33	50	5	54
3rd instar	5	1	99	4	95	1	0
	10	2	98	3	95	2	1
	15	3	97	1	96	6	4
	20	1	99	3	96	8	6
	30	2	98	9	89	3	9
	50	3	97	11	86	7	16
	100	8	92	42	50	2	49
4th instar	5	0	100	3	97	0	1
	10	2	98	1	97	1	2
	15	2	98	2	96	0	2
	20	0	100	2	98	2	2
	30	0	100	5	95	8	11
	50	1	99	13	86	1	13
	100	3	97	13	84	6	20
			Per 100 pupae at the start of the treatment				
Newly emerged pupae	2 000	—	—	2	98	2	0
	5 000	—	—	7	93	1	4
	10 000	—	—	11	89	1	8

[a] Larvae were exposed continuously until pupation, and the newly emerged pupae were exposed for 28 h.
[b] Using Abbot's formula.

the same dosage the butyl, methyl and ethyl esters resulted in 27%, 24% and 22% mortalities, respectively.

Moulting and structural abnormalities

Treatment with chemosterilant at the larval stage caused abnormalities during larval moults and also at pupation. At the time of pupation moulting started at the mid-thoracic region, or posteriorly; the pupae thus emerged with a larval head and a pupal body. Normal pupal development and some pupal abnormalities are shown in the accompanying figure (d, e & f). These abnormalities resulted in the death of the pupae. Treatment of larvae with tepa at 70 ppm at 4th instar resulted in the greatest number of such abnormal pupae—more than 20%. Adult emergence also showed certain abnormalities but the frequency of these was less than 5% in tepa

treatments. Emergence of adults in these cases did not start at the anterior end of the pupa as in normal pupae, but hatching was initiated in the thoracic region (g and h in the figure). Those adults derived from larvae treated with toxic doses of tepa and sometimes those treated with apholate showed deformed thorax and abdomen, reduced or deformed wings, curved and weak legs, and antennae twisted or without bristles (i in the figure). Such adults could not fly and died immediately. Metepa and the diaziridinyl compounds did not cause many structural abnormalities.

Effect on oviposition and fertility

The effects of chemosterilants, applied at the larval and pupal stages, on the fecundity and fertility of *C. p. fatigans* are given in Tables 4–8. The percentage control of reproduction, calculated by Chamberlain's formula, is taken as the criterion in assessing the over-all efficacy of the chemosterilants, as in this calculation both oviposition rate and the observed sterility have been taken into consideration (Chamberlain, 1962).

Among the larval treatments, followed by crosses between treated males and treated females, apholate was found to be the most effective chemosterilant. It produced about 96% sterility and 98.8% control of reproduction with the non-toxic dose of 10 ppm (Table 4). Apholate at 5 ppm caused 83% control of reproduction. Larval treatment with tepa was effective in decreasing the oviposition rate considerably, and produced about 72% and 86% control

TABLE 4

EFFECT OF APHOLATE ON OVIPOSITION, EGG HATCHABILITY AND CONTROL OF REPRODUCTION OF *C. P. FATIGANS* ADULTS EXPOSED AS EARLY SECOND INSTAR LARVAE AND NEWLY EMERGED PUPAE

Stage of treatment	Concentration (ppm)	Sex treated	No. of egg-rafts laid [a]	Total no. of eggs laid [a]	Hatch (%)	Control of reproduction (%)
Larval treatment	0 (control)	–	32	4 948	89.9	–
	5	Both	24	1 698	44.0	83.2
		Male	32	4 800	45.0	51.5
		Female	26	1 782	82.3	67.1
	10	Both	20	1 288	3.9	98.9
		Male	32	4 868	16.7	81.8
		Female	20	1 300	52.0	84.8
Pupal treatment	0 (control)	–	20	4 400	90.0	–
	1 000	Both	14	2 048	61.0	73.5
		Male	20	3 516	71.0	41.9
		Female	14	2 074	80.2	62.2
	5 000	Both	10	1 944	22.7	89.9
		Male	18	3 130	54.6	61.3
		Female	12	1 684	77.1	70.5
	10 000	Both	10	1 438	9.9	96.8
		Male	18	2 876	10.0	93.5
		Female	12	1 424	71.0	76.9

[a] Per 20 females.

TABLE 5

EFFECT OF TEPA ON OVIPOSITION, EGG HATCHABILITY AND CONTROL OF REPRODUCTION OF *C. P. FATIGANS* ADULTS EXPOSED AS EARLY SECOND INSTAR LARVAE AND NEWLY EMERGED PUPAE

Stage of treatment	Concentration (ppm)	Sex treated	No. of egg-rafts laid [a]	Total no. of eggs laid [a]	Hatch (%)	Control of reproduction (%)
Larval treatment	0 (control)	–	30	4 609	92.4	–
	7	Both	20	1 998	59.1	72.2
		Male	22	3 080	70.0	50.6
		Female	20	1 996	69.4	67.2
	14	Both	16	1 220	48.0	86.2
		Male	14	1 534	62.0	77.6
		Female	16	1 522	61.0	78.2
Pupal treatment	0 (control)	–	26	4 435	96.6	–
	716	Both	24	3 222	62.6	52.9
		Male	26	4 382	64.6	32.5
		Female	26	4 310	87.5	11.9
	3 580	Both	14	2 304	2.6	98.6
		Male	26	4 420	2.6	97.3
		Female	26	4 214	63.0	38.0
	7 160	Both	–	–	0	100
		Male	12	2 344	1.4	99.3
		Female	12	1 448	34.2	88.5

[a] Per 20 females.

of reproduction with 7 ppm and 14 ppm treatments, respectively (Table 5). Metepa at 5 ppm was the least effective chemosterilant. Larval treatment with metepa at 10 ppm and 50 ppm induced about 63% and 90% control of reproduction (Table 6).

When the diaziridinyl compounds were used for larval treatment, the methyl ester was the most effective, in crosses between treated males and treated females (Table 7). At 10 ppm it produced about 95% control of reproduction while the isopropyl ester caused 76%, the ethyl and butyl esters 65%, and the propyl ester 52% control of reproduction, respectively (Table 7).

Apholate induced high sterility in treated males whereas it did not induce sterility to the same extent in treated females (Table 4). However, in the latter, the oviposition rate was considerably lowered and this resulted in high control of reproduction. Males and females treated with tepa produced almost the same level of sterility, though the fecundity was much lower in females (Table 4). Treatment with metepa at 10 ppm did not have much effect either on males or females but at the toxic dose of 50 ppm it showed the same trend of results as apholate and tepa; in males it produced high sterility while in females it caused low oviposition rates (Table 6).

Treatment of pupa with tepa followed by crosses between treated males and treated females, produced about 98% sterility with the non-toxic dose of 3580 ppm and there was no egg laying when the dosage was raised to 7160 ppm (Table 5). Apholate

TABLE 6

EFFECT OF METEPA ON OVIPOSITION, EGG HATCHABILITY AND CONTROL OF REPRODUCTION OF *C. P. FATIGANS* ADULTS EXPOSED AS EARLY SECOND INSTAR LARVAE AND NEWLY EMERGED PUPAE

Stage of treatment	Concentration (ppm)	Sex treated	No. of egg-rafts laid [a]	Total no. of eggs laid [a]	Hatch (%)	Control of reproduction (%)
Larval treatment	0 (control)	–	28	4 270	95.1	–
	1	Both	28	4 002	94.8	6.8
		Male	26	4 200	95.0	1.9
		Female	28	4 060	95.0	5.1
	5	Both	26	3 816	90.0	15.5
		Male	26	4 260	90.0	5.7
		Female	28	4 001	91.0	10.4
	10	Both	18	1 980	75.4	63.3
		Male	28	3 526	81.1	29.2
		Female	18	2 112	87.7	54.5
	50	Both	20	1 738	21.9	90.7
		Male	28	3 520	40.2	65.1
		Female	18	1 734	75.2	67.9
Pupal treatment	0 (control)	–	28	4 684	88.9	–
	500	Both	28	4 601	81.4	10.0
		Male	28	4 673	84.5	5.2
		Female	28	4 603	88.1	2.6
	2 000	Both	28	4 600	75.0	17.5
		Male	28	4 684	79.0	11.1
		Female	28	4 614	86.9	3.7
	5 000	Both	20	3 596	70.0	39.5
		Male	24	4 211	69.9	29.2
		Female	24	3 886	85.6	20.2
	10 000	Both	20	3 292	4.9	96.1
		Male	24	4 108	14.9	85.3
		Female	24	3 684	83.2	26.4

[a] Per 20 females.

TABLE 7

EFFECT OF TEPA AND ITS SUBSTITUTED BIS (1-AZIRIDINYL) ALKOXYPHOSPHINE OXIDES ON OVIPOSITION, EGG HATCHABILITY AND CONTROL OF REPRODUCTION OF *C. P. FATIGANS* ADULTS EXPOSED AS EARLY SECOND INSTAR LARVAE.

Chemosterilant	Concentration (ppm)	Mortality (%)	No. of egg-rafts laid [a]	Total no. of eggs laid [a]	Hatch (%)	Control of reproduction (%)
Tepa	5	—	24	2 402	64.9	57.1
	10	16.0	20	1 800	50.0	75.3
Methyl ester	5	3.0	24	2 914	89.3	28.6
	10	33.0	8	418	37.8	95.7
Ethyl ester	5	7.0	16	1 806	77.8	61.4
	10	13.0	16	1 780	72.9	64.4
Propyl ester	5	1.0	26	2 994	74.6	38.7
	10	4.0	28	2 772	62.6	52.3
Isopropyl ester	5	23.0	14	1 646	78.7	64.4
	10	58.0	10	1 064	81.4	76.2
Butyl ester	5	0.0	20	2 458	89.4	38.0
	10	9.0	18	1 602	78.8	65.3

[a] Per 20 females.

was less effective than tepa as it produced only 90% sterility at 10 000 ppm (Table 4). Metepa at the non-toxic dose of 10 000 ppm was more effective than apholate, inducing about 95% sterility (Table 6). Among the diaziridinyl esters 10 000 ppm of the isopropyl and ethyl esters gave about 100% and 90% sterility, respectively, after pupal treatment (Table 8). The other chemosterilants of the same series produced only 21%–40% sterility in similar treatments.

Treatment of pupae with tepa induced higher sterility in treated males than in treated females (Table 5). Fecundity in treated females was lowered only at 7160 ppm. Similar results were obtained with metepa when used at 10 000 ppm (Table 6). Also when treated with apholate at 10 000 ppm, the

TABLE 8

EFFECT OF TEPA AND ITS SUBSTITUTED BIS (1-AZIRIDINYL) ALKOXYPHOSPHINE OXIDES ON OVIPOSITION, EGG HATCHABILITY AND CONTROL OF REPRODUCTION OF *C. P. FATIGANS* ADULTS EXPOSED AS NEWLY EMERGED PUPAE

Chemosterilant [a]	Mortality (%)	No. of egg-rafts laid [b]	Total no. of eggs laid [b]	Hatch (%)	Control of reproduction (%)
Tepa	65.0	0	0	0.0	100.0
Methyl ester	24.5	30	4 467	78.7	31.4
Ethyl ester	22.0	32	3 176	9.7	94.0
Propyl ester	9.0	32	5 314	76.6	20.7
Isopropyl ester	9.0	30	3 908	0.7	99.5
Butyl ester	27.0	30	4 100	58.9	52.9

[a] Pupae exposed for 28 h at 10 000 ppm.
[b] Per 20 females.

treated males were more effective than treated females in producing sterility when crossed with untreated mosquitos (Table 4). However, oviposition was lowered in the treated females.

Apholate and tepa at non-toxic doses induced structural abnormalities in eggs and the larvae of *C. p. fatigans*. Chemosterilized female mosquitos, crossed with either normal males or treated males, laid abnormal eggs. These eggs were spheroidal in shape and were smaller in size than the normal ones and sometimes had tapering ends which lacked pigmentation (a and b in the figure). Larval treatment with tepa at 14 ppm and pupal treatment with tepa at 3580 ppm produced about 2% and 2.6% of spherical abnormal eggs. Similarly larval treatment with apholate at 5 ppm and pupal treatment with apholate at 10 000 ppm resulted in little more than 1%–2% of such eggs. However, in the latter case, the number of unpigmented abnormal eggs was as high as 13.5% when treated females were crossed with normal males. Dissection of sterile eggs (normal and abnormal) showed fully developed larvae in a few eggs, many of which failed to hatch. In the apholate treatment, as shown in Table 9, the frequency of sterile eggs with larvae was greater when treated females were crossed with normal males, both after larval treatment and after pupal treatment.

The percentage hatch of abnormal eggs was as low as 5% and the larvae that hatched out from such eggs showed deformed heads, thoraces and posterior appendages. These larvae could not grow and never survived more than 96 h; most of them died soon after emergence.

TABLE 9

EFFECTS OF APHOLATE ON THE FREQUENCY OF STERILE EGGS BEARING LARVAE

	Apholate concentration (ppm)	Sex treated	Non-viable eggs with larvae (%)
Larval treatment	5	Both	10.0
		Males	8.2
		Females	38.1
	10	Both	5.0
		Males	4.7
		Females	29.1
Pupal treatment	5 000	Both	8.0
		Males	8.0
		Females	40.0
	10 000	Both	3.0
		Males	5.0
		Females	30.0

DISCUSSION

Aziridines are quite unstable compounds and the chemicals used in the present study may have varied in their stability in aqueous solution; their stability also varies with the temperature and the pH of the solution. In our experiments a constant temperature of 26.7°C was maintained and the pH of the rearing medium was neutral throughout.

Nevertheless the chemicals we used may have become partially or completely degraded during the treatments (from 2nd instar to 4th instar and to pupation) and thus it cannot be assumed that the conditions of treatment remained the same throughout all the stages of larval development. These factors should be taken into account when the results of our trials are assessed. Dame, Woodward & Ford (1964) found, however, that apholate and tepa, in aqueous solution with organic food materials, did not degrade under laboratory conditions for a period of 6 days and that 2 batches of larvae could be sterilized with the same solution.

Among the polyaziridinyl chemosterilants used in the present investigations, larval treatment with apholate was found to be the best in inducing almost 100% sterility with a non-toxic dose of 10 ppm. Metepa was least effective and tepa, though more effective than metepa in larval treatment, produced high mortality at various life stages of *C. p. fatigans*. Chang & Bořkovec (1964) found that tepa, on the contrary, was 13 times more active in sterilizing male houseflies than its methyl homologue, metepa. Apholate was shown to be superior to tepa and metepa in *C. p. quinquefasciatus* Say (= *C. p. fatigans*) after treatment of larvae or adults (Mulla, 1964). Apholate was also found to produce more sterility in adults of *C. p. quinquefasciatus* than tepa (McCray & Schoof, 1967) using mist application. With the same species Murray & Bickley (1964) found that larval treatment with 10 ppm and 15 ppm of apholate produced high sterility. Das (1967) also reports that treatment of larvae or adults with apholate induced high sterility in *C. p. fatigans*. But in *Aedes aegypti* (L.) tepa and apholate were equally active in producing sterility after larval treatment whereas after adult treatment apholate was more effective (Weidhaas, 1962; Dame, Woodward & Ford, 1964). In *Anopheles quadrimaculatus* Say, both apholate and tepa were equally

good after adult treatment (Weidhaas, Schmidt & Seabrook, 1962). However, in adult houseflies treatment with tepa was more effective than with apholate (Labrecque, 1961).

The methyl ester, among the diaziridinyl compounds, was more effective than tepa for larval treatment, though these compounds caused moderate toxicity in *C. p. fatigans*. The other substituted esters, except the isopropyl ester, were less effective than tepa in inducing sterility. With houseflies, all substitutions at the aziridinyl carbon atom of the tepa molecule decreased the sterilizing activity of the parent compound (Bořkovec, Woods & Brown, 1966). It is an interesting finding that in some cases the replacement of one aziridinyl moiety in tepa with alkoxy substituents induced the same or a higher level of sterility in *C. p. fatigans* as tepa.

With pupal treatment, tepa was found to be better, as a non-toxic dose could induce about 99% sterility. But apholate and metepa, even with higher doses, could not produce as much sterility as tepa. Among the diaziridinyl esters, the isopropyl and ethyl esters produced about 100% and 91% sterility, respectively, after pupal treatment, though these compounds were not as effective for larval treatment. The number of aziridinyl groups per molecule of the chemosterilant cannot be directly correlated with its sterilizing activity as some of the diaziridines were more active than triaziridines (Bořkovec, 1966). This is also evident from the present data, apholate with 6 aziridinyl rings being less effective for pupal treatment, and metepa being less active than some diaziridinyl esters for larval and pupal treatment. The present results also indicate that the sterilizing activity of each compound varied with the stages at which they were applied and hence structure–activity relationships could not be traced. Similarly the toxicity of the chemosterilants could not be correlated with the number of aziridinyl rings or types of substitutions. In *C. p. fatigans*, the sterilizing dose of tepa was moderately toxic in treatment of larvae but not so in treatment of pupae while apholate was toxic only with pupal treatment. These variations were shown by all the alkylating chemicals used in the present investigation. The toxicity and sterility produced by larval or pupal treatments with a chemosterilant probably depend on the rate of penetration, the duration of treatment and the mode of action of each chemosterilant. It is evident from the data that the stage at which the treatment is given is also important.

Pupal treatment of mosquitos is advantageous, since the pupae are tolerant to high doses of the chemosterilants and sterility can be induced with shorter duration of treatment. Also the mosquitos can be sexed more easily at the pupal stage and thus the treatment can be applied to either sex as required. White (1966) obtained complete sterility of *A. aegypti* with pupal treatment and Grover, Pillai & Dass (1967) also found that pupal treatment of *C. p. fatigans* with certain alkylating chemosterilants was effective. Knipling et al. (1968) considered that chemosterilization of mosquitos by pupal treatment was worth exploiting in sterilization programmes. Das (1967) did not find that pupal treatment induced much sterility in *C. p. fatigans*. This was evidently due to the low doses of apholate that were employed in the pupal treatments in his studies. In our pupal treatments females of *C. p. fatigans* required higher doses of chemosterilant to induce complete sterility. Similar results were obtained by White (1966) in *A. aegypti* and he suggested that this was probably due to the larger size of female pupae.

In general, both in larval and pupal treatments all the chemosterilants induced higher sterility in males than in females. However, in a few instances, the control of reproduction was greater in crosses using treated females than in those using treated males. In females the chemosterilants reduced considerably the rate of oviposition either due to the complete failure of the females to lay eggs or due to a reduction in number of eggs per raft. Das (1967) also found that the apholate treatment of larvae and adults produced more sterility in males and low fecundity in females.

It is now known that alkylating chemosterilants can inhibit DNA synthesis in the nuclei of follicular cells and nurse cells of the ovary in the stable-fly (Chamberlain & Barrett, 1968). This suggests that DNA-dependent protein synthesis during the gonadotrophic cycle of female mosquitos is inhibited and egg formation is thus prevented.

Treatment with tepa, and to a lesser extent with apholate, produced moulting and structural abnormalities. The structural syndromes may have been due to the mutagenic properties of tepa and apholate (Auerbach, 1958). These chemosterilants might also serve as metabolic poisons, although no experimental evidence for this has been produced so far. Tepa has been shown to cause copulatory aberrations and affected longevity of male cabbage loopers (Henneberry, Shorey & Kishaba, 1966). In *C. p. fatigans*, abnormalities were also induced

in the eggs and larvae of treated mosquitos. Similar abnormalities in eggs were observed in *A. aegypti* after pupal and adult treatment with thiotepa (Bertram, 1963; White, 1966). Smittle, Schmitt & Burden (1966) found structural abnormalities in embryos of the German cockroach due to treatment with tepa at the nymph and adult stages.

The exact mode of action of aziridinyl compounds is not fully understood. In males, the alkylation of chromatin material in the sperm nucleus has been suggested, but in females the mode of action is uncertain (Bořkovec, 1966). In the stable-fly, DNA synthesis in the ovary was found to be inhibited by apholate treatment (Chamberlain & Barrett, 1968). Apholate was found to cause visible chromosomal aberrations, such as breaks, deletion, clumping of chromatin, etc., in the somatic and reproductive organs of *A. aegypti* (Rai, 1964). Murray & Bickley (1964) reported reduction in the size of the testes in *C. p. quinquefasciatus* treated with apholate. Sperm depletion has been shown in case of apholate-sterilized males of *A. aegypti* (Sharma & Rai, 1967). Kilgore & Painter (1964) and Painter & Kilgore (1967) have shown inhibition of DNA synthesis in eggs of houseflies chemosterilized with apholate and thiotepa. The induction of sterility in the eggs may be because of induced dominant lethal genes, and the eggs would then fail to synthesize additional DNA after being laid. Dissection of non-viable eggs of *C. p. fatigans* resulting from a cross involving an apholate-treated male showed death of the embryos at an early stage of development. In crosses with treated females the effect was delayed and a high percentage of larvae were found in the eggs. Similar results were obtained by Fahmy & Fahmy (1954) and LaChance & Riemann (1964) in *Drosophila* and screw-worm flies, respectively, using tretamine.

The possibility of applying aziridinyl chemosterilants to natural breeding populations needs special caution, since these compounds are known to be carcinogenic as well as mutagenic. Furthermore, these compounds are highly unstable and break down into non-sterilizing products at high temperatures and in the presence of organic materials. Dame, Woodward & Ford (1964) examined the possibility of chemosterilant treatment of larval breeding waters and found that apholate coated on pyrophyllite at 15 ppm gave complete sterility, even when used over soil. In the laboratory both apholate (pyrophyllite formulation) and tepa were quite effective for a period of 6 days. In the field, the tepa solution became ineffective after 3 days, while apholate–pyrophyllite formulations were effective even after ageing for 1 week. Thus in field conditions tepa appeared to break down very quickly.

The treatment of pupae in their natural habitats is very difficult since pupae need very high concentrations. If a chemosterilant had to be used for treating natural aquatic populations, it would not be possible to treat all of the breeding places, but some of the main breeding sites could be dealt with. In field conditions, it would even be possible to use a partially toxic dose that would give very high sterility.

With *Culex p. fatigans*, which breeds in foul, stagnant water or sewage water, the feasibility of treating the breeding waters looks less practicable; however, it may be possible in this case to use sterilants mixed in a food bait diet or water with adult attractants. When natural populations are exposed to the sterilant, the percentage control of reproduction gives an exact idea of the total effect of the sterilant in decreasing the natural populations, since it takes into account the number of eggs laid by the treated female. If, however, the purpose of the treatment is to produce sterile males in the laboratory, which are later to be released in the field, the impact of the chemical on egg production by the female is only a matter of academic interest.

In the case of *Culex p. fatigans*, the release of laboratory-reared sterile males among natural populations seems more feasible than the treatment of natural populations with aziridinyl compounds. For such a release programme, it is essential to have a sterilant which can induce very high sterility in males at a non-toxic dose. In this context, treatment of pupae with aziridinyl compounds would seem a promising approach. Field trials on the effect of releasing sterilized males of *C. p. fatigans* into natural populations have already been attempted by Krishnamurthy, Ray & Joshi (1962), using irradiated males, and by Laven (1967) using incompatible males.

The present studies clearly indicate that many of the chemosterilants employed are very promising in sterilizing *C. p. fatigans* and that further studies on these compounds would be worth while.

ACKNOWLEDGEMENTS

We wish to thank Professor B. R. Seshachar, Head of the Department of Zoology, University of Delhi, India, for encouragement and Professor C. M. S. Dass for helpful discussions. We are also grateful to Dr A. B. Bořkovec, US Department of Agriculture, Beltsville, Md., USA, for providing the samples of chemosterilants.

RÉSUMÉ

CHIMIOSTÉRILISATION DE *CULEX PIPIENS FATIGANS* WIEDEMANN PAR EXPOSITION DES STADES AQUATIQUES: 1. POUVOIR STÉRILISANT DE CERTAINS COMPOSÉS AZIRIDINYLES

Les auteurs ont étudié l'activité stérilisante de huit composés aziridinyles sur le moustique domestique tropical *Culex pipiens fatigans* Wiedemann. Les produits utilisés comprenaient trois composés polyaziridinyles (apholate, metepa et tepa) et cinq composés alcoyloxydiazirinidyles avec comme substituants les groupements méthyle, éthyle, butyle, propyle et isopropyle.

Les moustiques ont été stérilisés soit par exposition aux composés depuis le deuxième stade larvaire jusqu'à la nymphose, soit par exposition des nymphes pendant 28 heures. Les composés diaziridinyles n'ont exercé qu'une action toxique modérée tant sur les larves que sur les nymphes et, dans les deux cas, c'est le metepa qui s'est révélé le moins toxique. La dose stérilisante d'apholate n'était toxique que pour les nymphes et la dose stérilisante de tepa n'était toxique que pour les larves. Le traitement a entraîné une certaine mortalité pendant la période critique de la mue. L'apholate et le tepa ont provoqué quelques anomalies de la structure et de la mue et l'on a également constaté chez les moustiques stérilisés des anomalies des œufs, des larves et des adultes.

L'application à des larves de doses non toxiques d'apholate a entraîné un taux de stérilité de 100%; le tepa, par contre, s'est révélé moins actif. Parmi les composés diaziridinyles, seul l'ester méthylique a pu produire la stérilité lorsqu'il était appliqué à des doses non toxiques au stade larvaire. Pour la stérilisation des nymphes, le tepa était plus actif que l'apholate et le metepa et ce dernier s'est révélé supérieur à l'apholate aux doses non toxiques. Les esters isopropylique et propylique ont également donné des taux de stérilité élevés chez les nymphes, mais les autres composés diaziridinyles n'ont pas paru très prometteurs. D'une manière générale, l'apholate et le metepa ainsi que, dans une certaine mesure, le tepa ont provoqué un taux élevé de stérilité chez les mâles traités lors du croisement avec des femelles normales et réduit la fécondité des femelles traitées puis croisées avec des mâles normaux. Les données rassemblées par les auteurs n'ont fait apparaître aucun rapport entre la structure des composés utilisés et leur action stérilisante. Leurs effets toxiques et leur pouvoir stérilisant variaient suivant le stade du cycle évolutif auquel ils étaient appliqués. La présente étude montre que nombre de composés aziridinyles semblent offrir des possibilités intéressantes pour la chimiostérilisation de *C. p. fatigans*.

REFERENCES

Auerbach, C. (1958) *Ann. N.Y. Acad. Sci.*, **68**, 731-748

Bertram, D. S. (1963) *Trans. roy. Soc. trop. Med. Hyg.*, **57**, 322-335

Bořkovec, A. B. (1964) *Residue Rev.*, **6**, 87-103

Bořkovec, A. B. (1966) *Advanc. Pest Control Res.*, **7**, 1-143

Bořkovec, A. B., Woods, C. W. & Brown, R. T. (1966) *J. med. Chem.*, **9**, 522-526

Chamberlain, W. F. (1962) *J. econ. Ent.*, **55**, 240-248

Chamberlain, W. F. & Barrett, C. C. (1968) *Nature (Lond.)*, **218**, 471-472

Chang, S. C. & Bořkovec, A. B. (1964) *J. econ. Ent.*, **57**, 488-490

Chang, S. C. & Bořkovec, A. B. (1966) *J. econ. Ent.*, **59**, 1359-1362

Dame, D. A. & Schmidt, C. H. (1964) *J. econ. Ent.*, **57**, 77-81

Dame, D. A., Woodward, D. B. & Ford, H. R. (1964) *Mosquito News*, **24**, 1-6

Das, M. (1967) *Bull. Wld Hlth Org.*, **36**, 949-954

Fahmy, O. G. & Fahmy, M. J. (1954) *J. Genetics*, **52**, 603-619

Grover, K. K., Pillai, M. K. K. & Dass, C. M. S. (1967) *Curr. Sci.*, **36**, 625-627

Henneberry, T. J., Shorey, H. H. & Kishaba, A. N. (1966) *J. econ. Ent.*, **59**, 573-576

Kilgore, W. W. & Painter, R. R. (1964) *Biochem. J.*, **92**, 353-357
Knipling, E. F. (1959) *Science*, **130**, 902-904
Knipling, E. F., Laven, H., Craig, G. B., Pal, R., Kitzmiller, J. B., Smith, C. N. & Brown, A. W. A. (1968) *Bull. Wld Hlth Org.*, **38**, 421-438
Kirshnamurthy, B. S., Ray, S. N. & Joshi, G. C. (1962) *Indian J. Malar.*, **16**, 365-373
Labrecque, G. C. (1961) *J. econ. Ent.*, **54**, 684-689
LaChance, L. E. & Riemann, J. G. (1964) *Mutation Res.*, **1**, 318-333
Laven, H. (1967) *Nature (Lond.)* **216**, 383-384
McCray, E. M., Jr. & Schoof, H. F. (1967) *J. econ. Ent.*, **60**, 60-63
Morlan, H. B., McCray, E. M. Jr. & Kilpatrick, J. W. (1962) *Mosquito News*, **22**, 295-300
Mulla, M. S. (1964) *Mosquito News*, **24**, 212-217
Murray, W. S. & Bickley, W. E. (1964) *Univ. Maryland Agric. Exptl Sta. Bull.*, No. 134
Painter, R. R. & Kilgore, W. W. (1967) *J. Insect Physiol.*, **13**, 1105-1118
Rai, K. S. (1964) *Biol. Bull. mar. biol. Lab., Woods Hole*, **127**, 119-131
Sharma, V. P. & Rai, K. S. (1967) *Canad. Ent.*, **99**, 116-118
Smith, C. N. (1967) *Bull. Wld Hlth Org.*, **36**, 633-635
Smittle, B. J., Schmitt, J. B. & Burden, G. S. (1966) *J. econ. Ent.*, **59**, 1419-1423
Weidhaas, D. E. (1962) *Nature (Lond.)*, **195**, 786-787
Weidhaas, D. E., Schmidt, C. H. & Seabrook, E. L. (1962) *Mosquito News*, **22**, 283-291
White, G. B. (1966) *Nature (Lond.)*, **210**, 1372-1373